Prepn. of stable, single phase soln. of water-in-oil emulsion - by addn. of microorganisms as conc. aq. soln. to crude oil or refined prod.

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• **Abstract :**
EP-409314 A Stable, single-phased solns. of water-in-oil microemulsions contg. (parts of) microorganisms are obtd. by adding to crude oil and/or a refined prod. an aq. conc. soln. of (parts of) microorganisms in such a way that the aq. soln. becomes solubilised in the oil or refined prod., giving a blend in the form of a stable, single-phased soln.
Pref. a capillary-active substance, opt. anionic, cationic, neutral or zwitterionic cpds., partic. 'Briji' (RTM), 'Tween' (RTM), 'Span' (RTM), lipids, e.g. lecithin, asolecithin, AOP and other sulphosuccinates, NH4 salts and oxyethylene cpds., is dissolved in the crude oil or refined prod. esp. in amt. of 0.1-30(0.5-15) wt.%. The microorganisms are those which reduce or oxidise S cpds., e.g. Thiobacillus ferroxidans, Sulfolobus acidocaldarius, Pseudomonas alkaligenes, P.janii, P.abikonensis and other Pseudomonas, E.coli, Alkaligenes denitrificans, Desulfovibrio desulfuricans or arthrobacter species, or a photosynthetic bacterium, e.g. Cyanobacteria, or animal or vegetable cells, partic. yeasts, which can demolish or transpose aromatic cpds., e.g. Saccharomyces cerevisiae or Candida utilis. The parts of the microorganisms are spores, heterocystes, mitochondria, microsomes or lysosomes.
USE/ADVANTAGE - Use of the solns. for removing S from and/or reducing the S content in coal or crude oil or an oil refinery prod., esp. from mineral oil, motor oil, naphtha, kerosene, or heavy or light fuel oil is claimed. Bacteria, yeast cells and other microorganisms do not decay rapidly in crude oil, e.g. are viable for some weeks. (12pp Dwg.No.0/4)rt

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(54) Stable, single-phased solutions of water-in-oil microemulsions derived from crude oil and allied products and which contain microorganisms and/or parts thereof.

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Description

This invention relates to stable, single-phased solutions of microorganism-containing water-in-oil microemulsions, which are obtained from crude oil or crude-oil derivatives.

In order to remove sulphur-containing products from crude oil, naphtha and derivatives, attempts have been made long since to find microbiological procedures. As microorganisms, as can be seen, for example in a comprehensive paper published in 1978 by Malik (ref. (1) at the end of the present specification), lend themselves Desulfovibrio desulfuricans, Arthrobacter sp., pseudomonas sp., Pseudomonas aeruginosa, Acinetobacter sp., Rhizobium sp.

Later, also Pseudomonas alcaligenes, Alcaligenes denitrificans, Solfobolus acidocaldarius, Thiobacillus ferroxidans have been proposed (ref.2-6).

The problem of removal of sulphur from crude oil is connected with that of removal of sulphur from coal, and the above cited literature references (1-6) and in other references (7,8) this subject matter is thoroughly discussed. A comprehensive article by Andrews and Maczuga discusses this problem.

Inasmuch as nearly all microorganisms, and thus also the ones referred to above, can survive in crude oil poorly, the rule is to work in a two-phased system, wherein the microorganisms are introduced into an aqueous phase which is immiscible with crude oil. The reaction takes place at the interface, so that it is necessary to renew such contact surfaces continuously with a vigorous stirring.

A new interesting paper on the argument of the biphasic systems has appeared recently (ref.6). In such case the authors use in the organic phase a surfactant (Tween 80, Reg.Trade Mark), which possesses the capability of building reversible micellae within organic solvents. They achieve thereby a significant success in removing sulphur from coal. The authors, however, warn that enzymatic preparations are much more efficient than the corresponding microorganisms as such (ref.6).

It would be an asset, of course, for the microbiological demolition, should one be enabled to work within a single homogeneous phase, rather than within a biphasic system. This means, however, to find conditions under which the microorganisms, scattered throughout the crude oil homogeneously, are present in solution.

The solubilization of water-soluble proteins and other biopolymers in organic solvents by the agency of reversible micellae or water-in-oil microemulsions, is known a few years since (ref.9,10).

Contrary to the normal aqueous micellae, the reversible micellae are formed in apolar solvents. To this end surfactants are employed, which form spheroidal aggregates, in which the polar heads of the molecules of the surfactant form a polar core. In such a core it is possible to solubilize water (Water pool). Whenever the water content in a ternary system is comparatively high, water-in-oil microemulsion is spoken of, and reversible micellae are no more mentioned.

However, in the commmon practice, the difference between the two fields has not been made quite clear.

The invention is illustrated by the accompanying drawings, wherein:

Fig.1 is a diagrammatical showing of (a) normal,ie aqueous micellae , and (b) reversible micellae;

Fig.2 is a diagrammatical showing of the introduction of a protein in the "water pool" (aqueous core) of reversible micellae;

Fig.3 depicts the difference between a bacteria-containing biphasic system (a) and the corresponding single-phase system (b), and

Fig.4 shows the stability of cells solubilized in crude oil by means of Asolecthin (65 mM) and water (1M) as explained in the examples.

The difference between the normal, ie aqueous, micellae (a) and the reversible micellae (b) is shown in Fig.1 of the accompanying drawings.

The water pool in the reversible micellae, or in the water-in-oil microemulsion, is of outstanding importance, because it becomes possible to dissolve biopolymers in such water droplets in a secondary solubilization process. Thermodynamically stable solutions are obtained, which are clear, and in which the enzymes retain their activity.

A graphic representation of the solubilization process referred to above is presented in Fig.2.

In recent years it has also been made known that E.coli bacteria and other small bacteria could be solubilized in the solvent isopropyl palmitate (IPP) by the agency of the surfactant Tween (reg.Trade Mark)(ref.11).

Within a solution of the capillary-active agent Tween 85 (Reg.trade Mark) in IPP, reversible micellae are formed at the outset, whereafter a small volume of a microorganism-containing aqueous solution was added. Whenever the concentration of the bacteria and/or the volume of water is not too high, the result of this procedure is a clear solution, in which viable and active bacteria can be detected.

The same group of searchers has subsequently solubilized also mitochondria in the same system (ref.12).

Later, it has been announced by a Group in Mexico (ref.13) that it is possible to solubilize spores, bacteria and yeast cells in toluene, and this with phospholipids as the surfactants, however with a restricted viability of the cells.

All the studies referred to above on bacteria in a homogeneous phase are restricted to few conventional organic solvents; crude oil and other naturally oc-

curring oils have not been mentioned heretofore.

The objective of the present invention is thus to improve the state of the art referred to above, and to provide stable, single-phased solutions of water-in-oil microemulsions which contain microorganisms and/or parts of microorganisms.

The invention, therefore, provides, according to one of its aspects, a stable, single-phased solution of a water-in-oil microemulsion which contains microorganisms, parts of microorganisms selected from spores, heterocysts, mitochondria, microsomes, lysosomes, or a mixture thereof, obtained by microspraying into crude oil, or a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, containing from 0,1% to 30% by weight, relative to the weight of the crude oil, or a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, of at least one surfactant, an aqueous concentrated solution of microorganisms, selected from spores, heterocysts, mitochondria, microsomes, lysosomes, or a mixture thereof, in an amount of from 0,001% to 100% by volume relative to the volume of the crude oil, or of a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or of a mixture thereof.

According to another aspect, the invention provides a process for preparing the solution referred to above, which comprises the step of of microspraying into crude oil, or a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, containing from 0,1% to 30% by weight, relative to the weight of the crude oil, or a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, of at least one surfactant, an aqueous concentrated solution of microorganisms, selected from spores, heterocysts, mitochondria, microsomes, lysosomes, or a mixture thereof, in an amount of from 0,001% to 100% by volume relative to the volume of the crude oil, or of a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or of a mixture thereof.

The main advantage of the present invention consists in that conditions have been found in which bacteria, yeast cells and other microorganisms can be solubilized in crude oil, that is in such a way they do not decay for longer times, independently of the selected system. The microorganisms are introduced in the form of an aqueous solution (eg with a microspray, the technique of the internal spraying), and the water is completely solubilized by the crude oil.

The situation in the case of the solubilization of proteins can be diagrammatically represented: see Fig.2. It is really surprising that the cells remain in solution since it would be forecast that they, due to their size should show a tendency towards sedimentation from the solution already after a short time, due to the gravity pull, and towards aggregation. Without being bound to any special theory, it is surmised that the stabilization of the microorganisms in solution is to be construed as a consequence of the formation of a microemulsion: the microorganisms, particularly the bacteria, which are present in the water droplets, are a component part of the water-in-oil microemulsion system, and clearly remain blocked in the organic solution as guest-compounds in the stable aggregates which are geometrically closed by the surfactant molecules.

Presumably, the bacteria are protected by a few water layers and by a layer of capillary-active agent molecules, whereby the solubility in an organic medium is made possible.

Fig.3 tenders a graphic representation, which, however is to be construed merely diagrammatic, inasmuch as accurate experimental data on the structure of the micellar aggregates of bacteria are not yet available.

The special difference in density between microorganisms and solvents, and the advantageous value of the increment of the count index, dn/dc, contribute to a degree towards the optical clarity and the reduction of the dispersion of light.

As outlined above, all the factors contributing towards the formation of clear solutions of microorganisms (bacteria and eukariotic cells), must be still closer investigated.

The solutions prepared according to this invention are stable, transparent and homogeneous single-phased systems.

It is important to emphasize that, in the solutions made according to this invention, contrary to the Kwang-Il Lee and Teh Fu Yen system (ref.6), no biphasic system is formed. According to Kwang-Il Lee et al., the bacteria are not solubilized in the micellar phase, but, rather, they are present in the aqueous phase (see Fig.3a). A diagrammatical showing of the difference between the two systems is reproduced in Fig.3.

It is likewise important to add that, under the conditions selected by Kwang-Il and Teh Fu, the bacteria cannot be conveyed in the supernatant phase, that is to say that in the a) system it is not possible to directly obtain a situation such as that corresponding to what is represented at b).

For these reasons the two procedures are substantially and radically different from one another.

According to the present invention, different types of bacteria are solubilized in crude-oil products, by the agency of different surfactants, eg Tween 85 and Asolecthin. In the absence of surfactants and/or water, no solubilization occurs; one obtains a suspen-

sion of cells, which segregate comparatively rapidly.

It had been established that, in the case of certain defined types of crude oil, which , as a rule, occur in the form of a black suspension and usually contain many compounds, surfactants should not be introduced, absolutely. Stated another way, it is permissible to add directly to the oil, without any special pretreatment,an aqueous microorganism-containing solution. Without being bound to any special theory, it is surmised that this circumstance is presumably to be attributed to the fact that crude oil already contains molecules which are similar to those of the surfactants.

This observation is of course very important from the biotechnological standpoint, because, on its basis, the potential process of the microbiological decomposition of crude oil would become much cheaper and simpler.

Water, however, must be added also in such a case.

In order that a single phase might be obtained, it is important that the volume of the added aqueous solution should not overtake the limits of the thermodynamic stability of the microemulsion system, or, stated alternatively, if too much water is added, a biphased system is obtained.

It has been quite surprisingly ascertained that many microorganisms, which are contained in the solutions prepared according to this invention, are in a position to carry out microbiological reactions even in an environment unfavourable to life, such as crude oil doubtless affords.

Thereby the basic principles are provided for carrying out microbiological processes in crude oil and in the products of its refining.

In a first stage of the programme, experiments have been conducted, the aim of which was to determine that bacterial cells can be directly solubilized in mineral oil or in naphtha, and that such single-phased systems are stable, that is, that they do not bring about any phase splitting, even when the system is not shaken. In a second stage, the viability of the microorganisms in such systems was investigated.

Both these stages of the programme are described hereinafter.

First stage: Preparation of a single-phased system

Typically, 500 mg of Tween 85 or 250 mg of Asolecthin were solubilized in 5 ml of a crude-oil product at room temperature and with vigorous stirring (10% or 5% weight/volume, respectively. The aqueous suspension of the cells was adjusted with an appropriate nutrient medium for the microorganism concerned, to a concentration (typically) of 10^8cells/ml. With a microspray a small volume of this solution (about 2% v/v) of the organic surfactant solution was added, and vigorously shaken (about 1600 rpm). Shaking was discontinued after a few minutes. With larger cells, a short ultra-sound treatment may shorten the shaking run. The solubilization of cells in crude oil without surfactant agent added follows in the abovementioned way.By varying the water concentration, it is possible to determine the limits for building a homogeneous phase.

It has been ascertained that in motor oil (Tellus 33, Shell) it is possible to solubilize up to about 1% of water (v/v); in the case of crude oil, it is possible to add up to the double volume of water,but it is to be mentioned that the opacity of the product hardly permits that a clear boundary may be detected.

In this manner the micellar solutions of motor oil and mineral oil contain from about 10^6 to 10^7 cells/ml (counted relative to the total volume).

It is possible to go beyond these limits, while still having a single-phased system, if a greater concentration of surfactant is employed, eg. in the case of Asolecthin, one can solubilize twice more water by doubling the concentration of the surfactant and, thereby, add more cells correspondingly.

In this connection, attention is also directed to the fact that, above a certain cell concentration, the solution becomes saturated, that is to say that the redundant cells will precipitate.

Obviously it is possible to operate also microbiologically under such conditions, but no solution is obtained any more, but a suspension. Such a system could be employed technologically, but it is necessary to shake vigorously; so as to keep all the cells in contact with the solvent, and so one falls into the situation of the biphasic system once again.

With the procedure as outlined above, the following microorganisms were investigated:

Bakers' yeast, pseudomonas sp., Sulfolobulus, Thiobacter sulfoxidans, Bac.subtilis, Arthrobacter spp.HAI, the details of which are reported in the examples.

All of these solutions remain stable, that is, there is no sign of phase splitting, and, moreover, no significant precipitation of the cells was observed along a few weeks.

Second stage: Determination of the viability of the microorganisms in crude oil products.

The objective of this work consists in investigating the viability of the microorganisms in the systems obtained in the above indicated way.

To this end, the activity of the microorganisms is tested on agar plates: the concentration of the viable cells is determined by smearing with a crude oil microemulsion, previously diluted with 0,9% aqueous NaCl, a measureable number of cells (about 100 per each Petri-dish). 100% viability corresponds to the cell concentration at the start (t=0).

Typical results are shown in Fig.4. It can be seen

that the different bacteria and cells differ from each other as to stability, but the viability in many cases is designated as very good. Details can be found in the description of the Figure or the examples.

The important features of the present invention can be summarized as follows:

A process is proposed, which makes it possible to dissolve microorganisms, preferably bacteria, in an aqueous phase in mineral oil, so as to obtain a single liquid phase, for which microorganisms do not precipitate during a long time. Surfactants are preferably used (eg Tween or lipids), which are solubilized in crude oil or in a product obtained by refining, where in the case of raw oil it is possible to work also without any addition of surfactants.

Contrary to other processes provided in the literature, the process proposed herein is characterized in that the microorganisms which are present in crude oil are in a microemulsion, which brings about an efficient contact with the solvent. Inasmuch as a single liquid phase is in the question, no stirring is potentially required to secure a reaction of the microorganisms with the compounds which are present in the crude oil.

The invention makes it possible to treat microbiologically a crude oil preparation under a stationary condition.

Among others, those microorganisms are solubilized in crude oil, which are capable of demolishing sulphur-containing products. Possible chemical demolition processes and the appertaining reactions are the target of further reasearch work.

It is moreover shown that the viability of the microorganisms can be extended for weeks, and that, during such a time, no significant precipitation of the cells can be observed.

EXAMPLES

EXAMPLE 1:

100 mg of yeast are suspended in 1 ml of nutrient medium (YPD, consisting of 1% yeast extract, 2% bacteropeptone, 2% glucose in water). 100 microlitres of the suspension are sprayed in 5 ml of crude oil and stirred at 1600 rpm for about half an hour, until obtaining a homogeneous phase.

EXAMPLE 2:

The yeast is processed as outlined above and the same volume is transferred into 5 ml of a solution of crude oil with 10% Tween 85, and stirred to homogeneousness just as in Example 1.

EXAMPLE 3:

The same procedure as in Example 1 is followed, with yeast in a solution of 250 mg of Asolecthin in 5 ml of crude oil.

EXAMPLE 4:

The same procedure as in Example 1 is adopted, with yeast in a solution of 250 mg of Asolecthin in 5 ml of Tellus 33 motor oil (Shell).

EXAMPLE 5:

The same procedure as in Example 1 is followed, with yeast in a solution of 250 mg Tween 85 in 2,5 ml of isopropylpalmitate, which is mixed with 2,5 ml of Tellus 33 motor oil (Shell).

EXAMPLE 6:

From a solution of 30 mg/ml of Pseudomonas sp. in a nutrient medium, 100 microliters are added to a solution of Asolecthin/crude oil.(Procedure as in Example 3).

EXAMPLE 7:

The same volume of a spore solution of the Bacillus subtilis is solubilized as in Example 6 or Example 1 in Asolecthin/crude oil.

EXAMPLES 8-10:

As described in Example 6, Arthrobacter spp. (grown for 2 days from butanol), Sulfolobus Acidocaldarius and Thiobacillus sulfoxidans can likewise be introduced.

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Claims

Claims for the following Contracting States : AT, BE, DE, DK, FR, GB, GR, IT, LU, NL, SE

1. A stable, single-phased solution of a water-in-oil microemulsion which contains microorganisms, parts of microorganisms selected from spores, heterocysts, mitochondria, microsomes, lysosomes, or a mixture thereof, obtained by microspraying into crude oil, or a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, containing from 0,1% to 30% by weight, relative to the weight of the crude oil, or a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, of at least one surfactant, an aqueous concentrated solution of microorganisms, selected from spores, heterocysts, mitochondria, microsomes, lysosomes, or a mixture thereof, in an amount of from 0,001% to 100% by volume relative to the volume of the crude oil, or of a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or of a mixture thereof.

2. Solution according to Claim 1, wherein said surfactant is contained in said crude oil, or said product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, in an amount of from 0,15% to 15% by weight.

3. Solution according to Claim 1, wherein the surfactant is selected from the anionic, cationit, neutral and zwitter-ionic surfactants.

4. Solution according to Claim 1, wherein said surfactant is a sorbitan trioleate polyoxyalkylene derivative.

5. Solution according to to Claim 1, wherein said microorganisms are selected from bacteria and yeasts.

6. Solution according to Claim 5, wherein said bacteria possess a reducing or an oxidizing action towards sulphur-containing products.

7. Solution according to Claim 5, wherein said yeasts possess a demolishing activity or a transposition capability towards aromatic compounds.

8. Solution according to Claim 7, wherein the yeast is selected from Saccharomyces cerevisiae and Candida utilis.

9. Solution according to Claim 1, further containing at least one co-surfactant selected from fatty acids, alcohols and halogen-containing compounds.

10. Solution according to Claim 9, wherein said co-surfactant is present in an amount of from 0.1% to 100% by weight relative to the amount of the surfactant.

11. Solution according to Claim 1, wherein said aqueous solution additionally contains nutrients and salts for the microorganims.

12. Solution according to Claim 1, wherein said crude oil, or said product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, is blended with an organic solvent or a vegetable oil.

13. Solution according to Claim 12, wherein said organic solvent is selected from aromatic hydrocarbons, aliphatic hydrocarbons, fatty acid esters, alcohols, and halogen-substituted compounds.

14. Solution according to Claim 13, wherein said organic solvent is selected from benzene, toluene, cresol, pentane, octane, dodecane, fluorinated compounds and perfluorinated compounds.

15. Solution according to Claim 12, wherein said vegetable oil is selected from soybean seed oil, sunflowerseed oil, colza seed oil and olive oil.

16. Solution according to Claim 1, wherein said organic solvent or vegetable oil is present in an amount of from 1% to 1000% by volume relative to the volume of the crude oil, or of a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or of a mixture thereof.

17. A process for preparing a stable, single-phased solution of a water-in-oil microemulsion which contains microorganisms, parts of microorganisms selected from spores, heterocysts, mitochondria, microsomes, lysosomes, or a mixture thereof, comprising the step of microspraying into crude oil, or a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, containing from 0,1% to 30% by weight, relative to the weight of the crude oil, or a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, of at least one surfactant, an aqueous concentrated solution of microorganisms, selected from spores, heterocysts, mitochondria, microsomes, lysosomes, or a mixture thereof, in an amount of from 0,001% to 100% by volume relative to the volume of the crude oil, or of a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or of a mixture thereof.

Claims for the following Contracting State : ES

1. A process for preparing a stable, single-phased solution of a water-in-oil microemulsion which contains microorganisms, parts of microorganisms selected from spores, heterocysts, mitochondria, microsomes, lysosomes, or a mixture thereof, comprising the step of microspraying into crude oil, or a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, containing from 0,1% to 30% by weight, relative to the weight of the crude oil, or a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, of at least one surfactant, an aqueous concentrated solution of microorganisms, selected from spores, heterocysts, mitochondria, microsomes, lysosomes, or a mixture thereof, in an amount of from 0,001% to 100% by volume relative to the volume of the crude oil, or of a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or of a mixture thereof.

2. Process according to Claim 1, characterized in that said surfactant is contained in said crude oil, or said product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, in an amount of from 0,15% to 15% by weight.

3. Process according to Claim 1, characterized in that the surfactant is selected from the anionic, cationic, neutral and zwitter-ionic surfactants.

4. Process according to Claim 1, characterized in that said surfactant is a sorbitan trioleate polyoxyalkylene derivative.

5. Process according to to Claim 1, characterized in that said microorganisms are selected from bacteria and yeasts.

6. Process according to Claim 5, characterized in that said bacteria possess a reducing or an oxidizing action towards sulphur-containing products.

7. Process according to Claim 5, characterized in that said yeasts possess a demolishing activity or a transposition capability towards aromatic compounds.

8. Process according to Claim 7, characterized in that the yeast is selected from <u>Saccharomyces cerevisiae</u> and <u>Candida utilis.</u>

9. Process according to Claim 1, characterized in that at least one co-surfactant selected from fatty acids, alcohols and halogen-containing compounds is added to said crude oil, or said product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof.

10. Process according to Claim 9, characterized in that said co-surfactant is present in an amount of from 0.1% to 100% by weight relative to the amount of the surfactant.

11. Process according to Claim 1, characterized in that said aqueous solution additionally contains nutrients and salts for the microorganims.

12. Process according to Claim 1, characterized in that said crude oil, or said product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or a mixture thereof, is blended with an organic solvent or a vegetable oil.

13. Process according to Claim 12, characterized in that said organic solvent is selected from aromatic hydrocarbons, aliphatic hydrocarbons, fatty acid esters, alcohols, and halogen-substituted compounds.

14. Process according to Claim 13, characterized in that said organic solvent is selected from benzene, toluene, cresol, pentane, octane, dode-

cane, fluorinated compounds and perfluorinated compounds.

15. Process according to Claim 12, characterized in that said vegetable oil is selected from soybean seed oil, sunflowerseed oil, colza seed oil and olive oil.

16. Process according to Claim 1, characterized in that said organic solvent or vegetable oil is present in an amount of from 1% to 1000% by volume relative to the volume of the crude oil, or of a product of refining crude oil, selected from mineral oils, motor oils, naphtha, kerosene, light fuel oil and heavy fuel oil, or of a mixture thereof.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, DE, DK, FR, GB, GR, IT, LU, NL, SE

1. Stabile, einphasige Lösung einer Wasser-in-Öl-Mikroemulsion, die Mikroorganismen, Teile von Mikroorganismen, ausgewählt unter Sporen, Heterozysten, Mitochondrien, Mikrosomen, Lysosomen oder ein Gemisch hievon enthält, erhalten durch Mikrosprühen einer wäßrigen konzentrierten Lösung von Mikroorganismen, ausgewählt unter Sporen, Heterozysten, Mitochondrien, Mikrosomen, Lysosomen oder einem Gemisch hievon, in Rohöl oder eines Produktes der Rohölraffination, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon, mit einem Gehalt an 0,1 bis 30 Gew.-%, bezogen auf das Gewicht des Rohöls oder eines Produktes der Rohölraffination, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon, wenigstens eines grenzflächenaktiven Mittels, in einer Menge von 0,001 bis 100 Vol.-%, bezogen auf das Volumen des Rohöls oder eines Raffinationsproduktes von Rohöl, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon.

2. Lösung nach Anspruch 1, worin das grenzflächenaktive Mittel in dem Rohöl oder dem Rohöl-Raffinationsprodukt, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon, in einer Menge von 0,15 bis 15 Gew.-% enthalten ist.

3. Lösung nach Anspruch 1, worin das grenzflächenaktive Mittel unter anionischen, kationischen, neutralen und zwitterionischen grenzflächenaktiven Mitteln ausgewählt ist.

4. Lösung nach Anspruch 1, worin das grenzflächenaktive Mittel ein Sorbitantrioleat-Polyoxyalkylenderivat ist.

5. Lösung nach Anspruch 1, worin die Mikroorganismen unter Bakterien und Hefen ausgewählt sind.

6. Lösung nach Anspruch 5, worin die Bakterien gegenüber schwefelhältigen Produkten eine reduzierende oder eine oxidierende Wirkung aufweisen.

7. Lösung nach Anspruch 5, worin die Hefen gegenüber aromatischen Verbindungen eine Abbauaktivität oder ein Transpositionsvermögen zeigen.

8. Lösung nach Anspruch 7, worin die Hefe unter Saccharomyces cerevisiae und Candida utilis ausgewählt ist.

9. Lösung nach Anspruch 1, mit einem weiteren Gehalt an wenigstens einem Co-Surfactant, ausgewählt unter Fettsäuren, Alkoholen und halogenhältigen Verbindungen.

10. Lösung nach Anspruch 9, worin das Co-Surfactant in einer Menge von 0,1 bis 100 Gew.-%, bezogen auf die Menge des grenzflächenaktiven Mittels, vorhanden ist.

11. Lösung nach Anspruch 1, worin die wäßrige Lösung zusätzlich Nährstoffe und Salze für die Mikroorganismen enthält.

12. Lösung nach Anspruch 1, worin das Rohöl oder das Rohöl-Raffinationsprodukt, ausgewählt unter Mineralölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon, mit einem organischen Lösungsmittel oder einem Pflanzenöl vermischt ist.

13. Lösung nach Anspruch 12, worin das organische Lösungsmittel unter aromatischen Kohlenwasserstoffen, aliphatischen Kohlenwasserstoffen, Fettsäureestern, Alkoholen und halogensubstituierten Verbindungen ausgewählt ist.

14. Lösung nach Anspruch 13, worin das organische Lösungsmittel unter Benzol, Toluol, Kresol, Pentan, Octan, Dodecan, fluorierten Verbindungen und perfluorierten Verbindungen ausgewählt ist.

15. Lösung nach Anspruch 12, worin das Pflanzenöl unter Sojabohnenöl, Sonnenblumenöl, Rapsöl und Olivenöl ausgewählt ist.

16. Lösung nach Anspruch 1, worin das organische Lösungsmittel oder das Pflanzenöl in einer Menge von 1 bis 1.000 Vol.-%, bezogen auf das Volumen des Rohöls oder eines Rohöl-Raffinationsproduktes, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon, vorliegt.

17. Verfähren zur Herstellung einer stabilen, einphasigen Lösung einer Wasser-in-Öl-Mikroemulsion, die Mikroorganismen oder Teile von Mikroorganismen, ausgewählt unter Sporen, Heterozysten, Mitochondrien, Mikrosomen, Lysosomen oder einem Gemisch hievon, enthält, umfassend die Stufe des Mikroversprühens einer wäßrigen konzentrierten Lösung von Mikroorganismen, ausgewählt unter Sporen, Heterozysten, Mitochondrien, Mikrosomen, Lysosomen oder einem Gemisch hievon, in Rohöl oder einem Rohöl-Raffinationsprodukt, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon, mit einem Gehalt an 0,1 bis 30 Gew.-%, bezogen auf das Gewicht des Rohöls oder eines Rohöl-Raffinationsproduktes, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon, wenigstens eines grenzflächenaktiven Mittels, in einer Menge von 0,001 bis 100 Vol.-%, bezogen auf das Volumen des Rohöls oder eines Rohöl-Raffinationsproduktes, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon.

Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zur Herstellung einer stabilen, einphasigen Lösung einer Wasser-in-Öl-Mikroemulsion, die Mikroorganismen oder Teile von Mikroorganismen, ausgewählt unter Sporen, Heterozysten, Mitochondrien, Mikrosomen, Lysosomen oder einem Gemisch hievon, enthält, umfassend die Stufe des Mikroversprühens einer wäßrigen konzentrierten Lösung von Mikroorganismen, ausgewählt unter Sporen, Heterozysten, Mitochondrien, Mikrosomen, Lysosomen oder einem Gemisch hievon, in Rohöl oder einem Rohöl-Raffinationsprodukt, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon, mit einem Gehalt an 0,1 bis 30 Gew.-%, bezogen auf das Gewicht des Rohöls oder eines Rohöl-Raffinationsproduktes, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon, wenigstens eines grenzflächenaktiven Mittels, in einer Menge von 0,001 bis 100 Vol.-%, bezogen auf das Volumen des Rohöls oder eines Rohöl-Raffinationsproduktes, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das grenzflächenaktive Mittel in dem Rohöl oder dem Rohöl-Raffinationsprodukt, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon, in einer Menge von 0,15 bis 15 Gew.-% enthalten ist.

3. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das grenzflächenaktive Mittel unter anionischen, kationischen, neutralen und zwitterionischen grenzflächenaktiven Mitteln ausgewählt ist.

4. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das grenzflächenaktive Mittel ein Sorbitantrioleat-Polyoxyalkylenderivat ist.

5. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die Mikroorganismen unter Bakterien und Hefen ausgewählt sind.

6. Verfahren nach Anspruch 5, dadurch gekennzeichnet, daß die Bakterien gegenüber schwefelhältigen Produkten eine reduzierende oder eine oxidierende Wirkung aufweisen.

7. Verfahren nach Anspruch 5, dadurch gekennzeichnet, daß die Hefen gegenüber aromatischen Verbindungen eine Abbauaktivität oder ein Transpositionsvermögen zeigen.

8. Verfahren nach Anspruch 7, dadurch gekennzeichnet, daß die Hefe unter Saccharomyces cerevisiae und Candida utilis ausgewählt wird.

9. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß dem Rohöl oder Rohöl-Raffinationsprodukt, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon, wenigstens ein Co-Surfactant, ausgewählt unter Fettsäuren, Alkoholen und halogenhältigen Verbindungen, zugesetzt wird.

10. Verfahren nach Anspruch 9, dadurch gekennzeichnet, daß das Co-Surfactant in einer Menge von 0,1 bis 100 Gew.-%, bezogen auf die Menge des grenzflächenaktiven Mittels, vorhanden ist.

11. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die wäßrige Lösung zusätzlich Nährstoffe und Salze für die Mikroorganismen enthält.

12. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das Rohöl oder das Rohöl-Raffinationsprodukt, ausgewählt unter Mineralölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon, mit einem organischen Lösungsmittel oder einem Pflanzenöl vermischt ist.

13. Verfahren nach Anspruch 12, dadurch gekennzeichnet, daß das organische Lösungsmittel unter aromatischen Kohlenwasserstoffen, aliphatischen Kohlenwasserstoffen, Fettsäureestern, Alkoholen und halogensubstituierten Verbindungen ausgewählt ist.

14. Verfahren nach Anspruch 13, dadurch gekennzeichnet, daß das organische Lösungsmittel unter Benzol, Toluol, Kresol, Pentan, Octan, Dodecan, fluorierten Verbindungen und perfluorierten Verbindungen ausgewählt ist.

15. Verfahren nach Anspruch 12, dadurch gekennzeichnet, daß das Pflanzenöl unter Sojabohnenöl, Sonnenblumenöl, Rapsöl und Olivenöl ausgewählt ist.

16. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das organische Lösungsmittel oder das Pflanzenöl in einer Menge von 1 bis 1.000 Vol.-%, bezogen auf das Volumen des Rohöls oder eines Rohöl-Raffinationsproduktes, ausgewählt unter Mineralölen, Motorölen, Naphtha, Kerosin, leichtem Heizöl und schwerem Heizöl oder einem Gemisch hievon, vorliegt.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, DE, DK, FR, GB, GR, IT, LU, NL, SE

1. Solution stable, à une seule phase, d'une microémulsion eau dans huile, qui contient des microorganismes, des parties de microorganismes choisies parmi les spores, les hétérocystes, les mitochondries, les microsomes, les lysosomes, ou un mélange des précédents, que l'on obtient par micropulvérisation dans du pétrole brut ou dans un produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou un mélange des précédents, contenant de 0,1 % à 30 % en poids, par rapport au poids du pétrole brut ou du produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou un mélange des précédents, d'au moins un tensio-actif, d'une solution aqueuse concentrée de microorganismes choisis parmi les spores, les hétérocystes, les mitochondries, les microsomes, les lysosomes, ou un mélange des précédents, en une proposition de 0,001 % à 100 % en volume par rapport au volume du pétrole brut ou du produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou d'un mélange des précédents.

2. Solution selon la revendication 1, dans laquelle ledit tensio-actif est présent dans ledit pétrole brut ou ledit produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou un mélange des précédents, en une proportion de 0,15 % à 15 % en poids.

3. Solution selon la revendication 1, dans laquelle le tensio-actif est choisi parmi les tensio-actifs anioniques, cationiques, neutres ou zwitterioniques.

4. Solution selon la revendication 1, dans laquelle ledit tensio-actif est un dérivé polyoxyalkylène de trioléate de sorbitane.

5. Solution selon la revendication 1, dans laquelle lesdits microorganismes sont choisis parmi les bactéries et les levures.

6. Solution selon la revendication 5, dans laquelle lesdites bactéries ont une action réductrice ou oxydante sur les produits sulfurés.

7. Solution selon la revendication 5, dans laquelle lesdites levures ont une action destructrice vis-à-vis des composés aromatiques ou sont aptes à réaliser une transposition des composés aromatiques.

8. Solution selon la revendication 7, dans laquelle la levure est choisie parmi Saccharomyces cerevisiae et Candida utilis.

9. Solution selon la revendication 1, contenant en outre au moins un co-tensio-actif choisi parmi les acides gras, les alcools et les dérivés halogénés.

10. Solution selon la revendication 9, dans laquelle ledit co-tensio-actif est présent en une proportion de 0,1 % à 100 % en poids par rapport à la quan-

tité du tensio-actif.

11. Solution selon la revendication 1, dans laquelle ladite solution aqueuse contient en plus des substances nutritives et des sels pour les microorganismes.

12. Solution selon la revendication 1, dans laquelle ledit pétrole brut ou ledit produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou un mélange des précédents, est mélangé avec un solvant organique ou une huile végétale.

13. Solution selon la revendication 12, dans laquelle ledit solvant organique est choisi parmi les hydrocarbures aromatiques, les hydrocarbures aliphatiques, les esters d'acide gras, les alcools et les dérivés halogénés.

14. Solution selon la revendication 13, dans laquelle ledit solvant organique est choisi parmi le benzène, le toluène, le crésol, le pentane, l'octane, le dodécane, les composés fluorés et les composés perfluorés.

15. Solution selon la revendication 12, dans laquelle ladite huile végétale est choisie parmi l'huile de graine de soja, l'huile de graine de tournesol, l'huile de graine de colza et l'huile d'olive.

16. Solution selon la revendication 1, dans laquelle ledit solvant organique ou ladite huile végétale est présent en une proportion de 1 % à 1000 % en volume par rapport au volume du pétrole brut, ou du produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou d'un mélange des précédents.

17. Procédé de préparation d'une solution stable, à une seule phase, d'une micro-émulsion eau dans huile, qui contient des microorganismes, des parties de microorganismes choisies parmi les spores, les hétérocystes, les mitochondries, les microsomes, les lysosomes, ou un mélange des précédents, comprenant l'étape consistant à effectuer la micropulvérisation dans du pétrole brut, ou un produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou un mélange des précédents, contenant de 0,1 % à 30 % en poids, par rapport au poids du pétrole brut ou du produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou d'un mélange des précédents, d'au moins un tensio-actif, d'une solution aqueuse concentrée de microorganismes, choisis parmi les spores, les hétérocystes, les mitochondries, les microsomes, les lysosomes, ou un mélange des précédents, en une proportion de 0,001 % à 100 % en volume par rapport au volume du pétrole brut ou du produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteur, le naphta, le kérosène, le fuel léger et le fuel lourd, ou d'un mélange des précédents.

Revendications pour l'Etat contractant suivant : ES

1. Procédé de préparation d'une solution stable, à une seule phase, d'une micro-émulsion eau dans huile, qui contient des microorganismes, des parties de microorganismes choisies parmi les spores, les hétérocystes, les mitochondries, les microsomes, les lysosomes, ou un mélange des précédents, comprenant l'étape consistant à effectuer la micropulvérisation dans du pétrole brut, ou un produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou un mélange des précédents, contenant de 0,1 % à 30 % en poids, par rapport au poids du pétrole brut ou du produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou d'un mélange des précédents, d'au moins un tensio-actif, d'une solution aqueuse concentrée de microorganismes, choisis parmi les spores, les hétérocystes, les mitochondries, les microsomes, les lysosomes, ou un mélange des précédents, en une proportion de 0,001 % à 100 % en volume par rapport au volume du pétrole brut ou du produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteur, le naphta, le kérosène, le fuel léger et le fuel lourd, ou d'un mélange des précédents.

2. Procédé selon la revendication 1, caractérisé en ce que ledit tensio-actif est présent dans ledit pétrole brut ou ledit produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou un mélange des précédents, en une proportion de 0,15 % à 15 % en poids.

3. Procédé selon la revendication 1, caractérisé en ce que le tensio-actif est choisi parmi les tensioactifs anioniques, cationiques, neutres ou zwitterioniques.

4. Procédé selon la revendication 1, caractérisé en

ce que ledit tensio-actif est un dérivé polyoxyalkylène de trioléate de sorbitane.

5. Procédé selon la revendication 1, caractérisé en ce que ledit microorganismes sont choisis parmi les bactéries et les levures.

6. Procédé selon la revendication 5, caractérisé en ce que lesdites bactéries ont une action réductrice ou oxydante sur les produits sulfurés.

7. Procédé selon la revendication 5, caractérisé en ce que lesdites levures ont une action destructrice vis-à-vis des composés aromatiques ou sont aptes à réaliser une transposition des composés aromatiques.

8. Procédé selon la revendication 7, caractérisé en ce que la levure est choisie parmi Saccharomyces cerevisiae et Candida utilis.

9. Procédé selon la revendication 1, caractérisé en ce qu'au moins un co-tensio-actif, choisi parmi les acides gras, les alcools et les dérivés halogénés, est ajouté audit pétrole brut ou audit produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou à un mélange des précédents.

10. Procédé selon la revendication 9, caractérisé en ce que ledit co-tensio-actif est présent en une proportion de 0,1 % à 100 % en poids par rapport à la quantité du tensio-actif.

11. Procédé selon la revendication 1, caractérisé en ce que ladite solution aqueuse contient en plus des substances nutritives et des sels pour les microorganismes.

12. Procédé selon la revendication 1, caractérisé en ce que ledit pétrole brut ou ledit produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou un mélange des précédents, est mélangé avec un solvant organique ou une huile végétale.

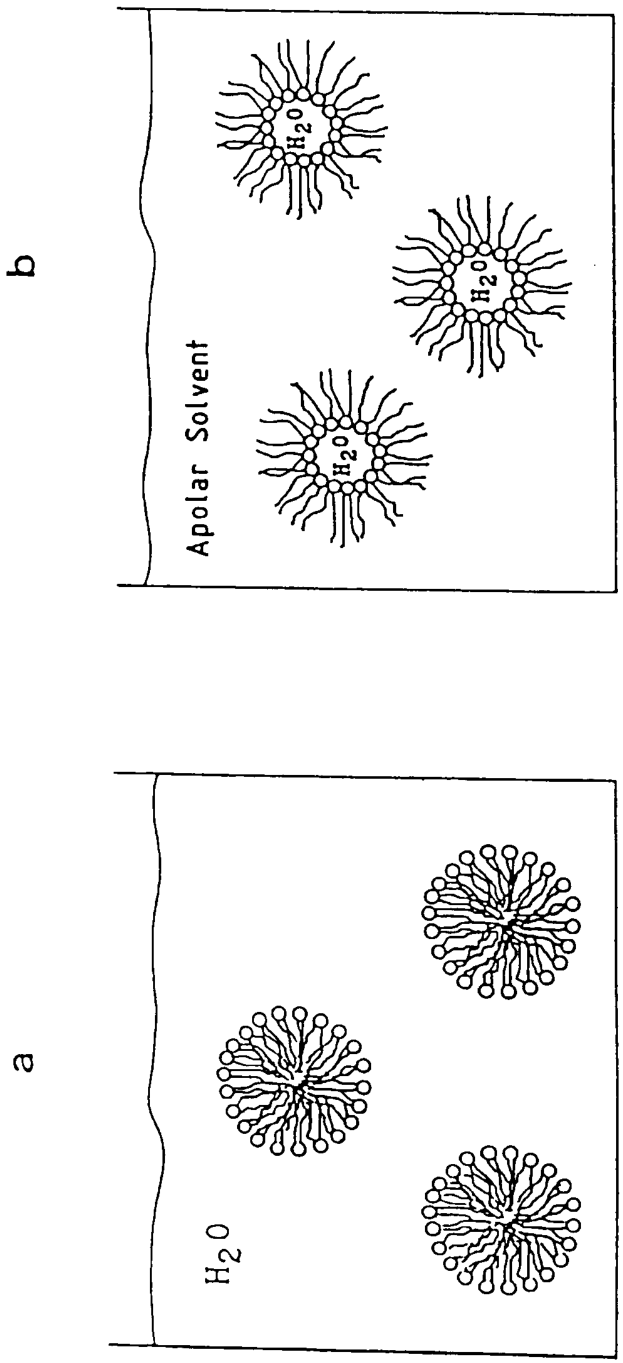
13. Procédé selon la revendication 12, caractérisé en ce que ledit solvant organique est choisi parmi les hydrocarbures aromatiques, les hydrocarbures aliphatiques, les esters d'acide gras, les alcools et les dérivés halogénés.

14. Procédé selon la revendication 13, caractérisé en ce que ledit solvant organique est choisi parmi le benzène, le toluène, le crésol, le pentane, l'octane, le dodécane, les composés fluorés et les composés perfluorés.

15. Procédé selon la revendication 12, caractérisé en ce que ladite huile végétale est choisie parmi l'huile de graine de soja, l'huile de graine de tournesol, l'huile de graine de colza et d'huile d'olive.

16. Procédé selon la revendication 1, caractérisé en ce que ledit solvant organique ou ladite huile végétale est présent en une proportion de 1 % à 1000 % en volume par rapport au volume du pétrole brut, ou du produit de raffinage du pétrole brut, choisi parmi les huiles minérales, les huiles à moteurs, le naphta, le kérosène, le fuel léger et le fuel lourd, ou d'un mélange des précédents.

Fig.1



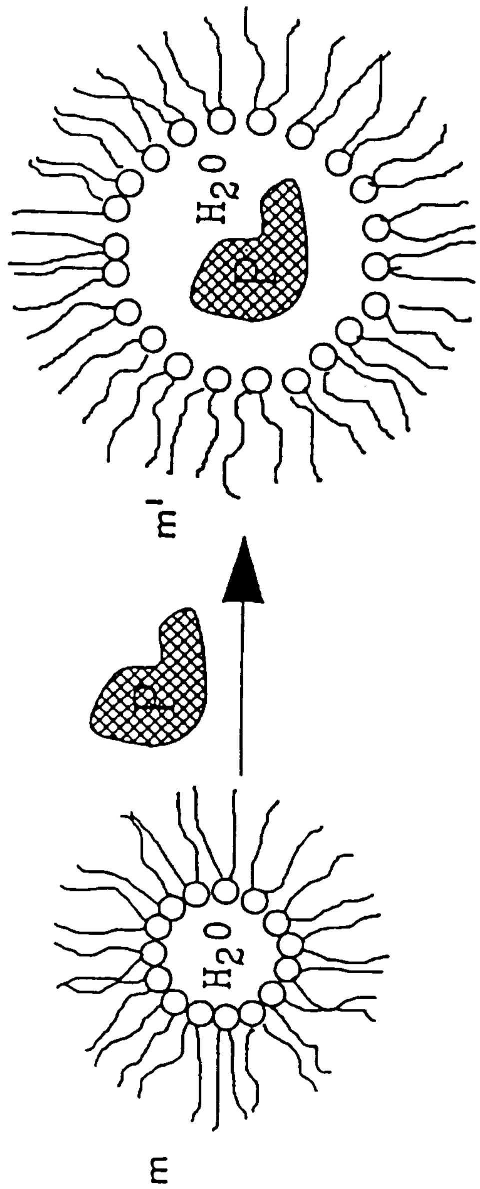
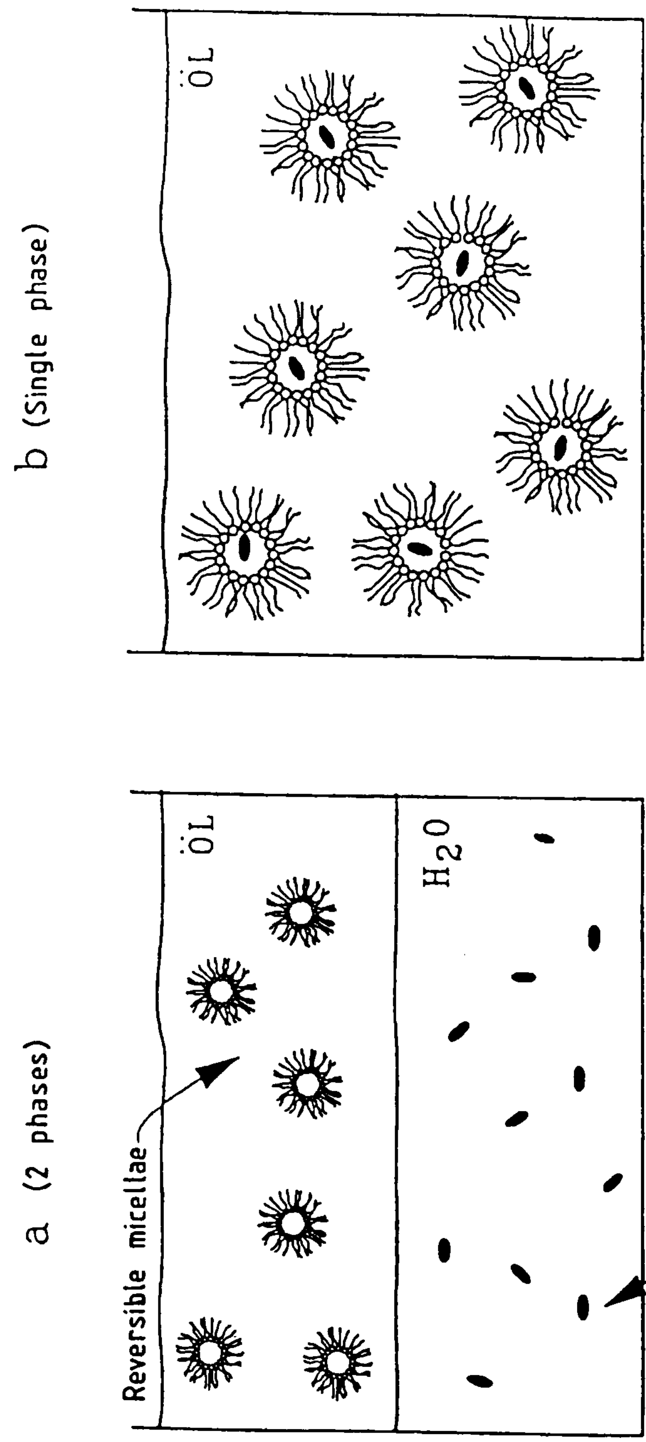


Fig.2

Fig.3


a (2 phases)
b (Single phase)
ÖL
ÖL
H_2O
Reversible micellae
Bacteria

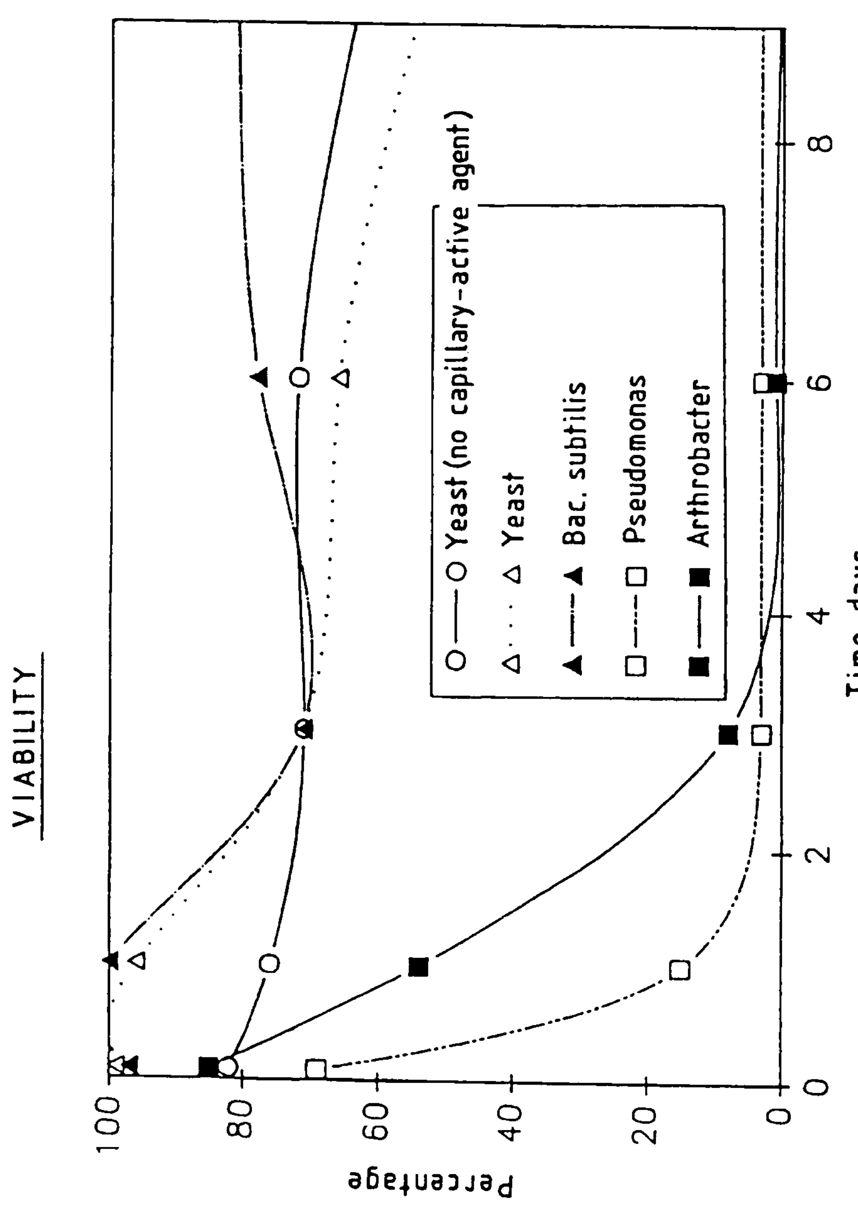

VIABILITY
Percentage
100
80
60
40
20
0
Time, days
0
2
4
6
8
Yeast (no capillary-active agent)
Yeast
Bac. subtilis
Pseudomonas
Arthrobacter

Fig.4